NEXT-GENERATION MATERIALS FOR BIOLOGICAL WEAPON DETECTION

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Introduction

As evidenced in the Gulf War, U.S. soldiers confronting an adversary owning chemical and biological weapons could face an attack by these weapons. Biological warfare (BW) has a long history, dating back to attempts by the Romans to foul their enemies' water supplies, and represents an important asymmetrical strategic threat to U.S. military forces and civilian populations. Unlike other weapons of mass effect, pathogenic viruses and bacteria and infectious proteins such as prions (the presumed causative factor in mad cow disease) are self-replicating; hence, the effects of many BW agents are amplified by secondary infection. Rapid, sensitive, and accurate detection and identification is therefore a priority in developing sensor technology that will meet the operational requirements of the future Army.

This article describes the Army's application of advanced biotechnology to devise new molecules that recognize BW agents, with an emphasis on how these molecules will provide the means to develop more specific and sensitive sensors to detect BW agents. It also addresses logistics issues such as cost-efficient manufacturing processes and improvements in total process quality assurance/quality control (QA/QC) in the life-cycle management of critical reagents. As used in this article, the term reagent refers to the engineered antibodies that specifically bind with each target BW agent.

Lessons From Nature

The tremendous complexity of most living things, including BW agents, provides a rich supply of unique molecules that can be used as distinctive signatures for an organism or class of organisms. Some sensors under development use sequences in the genetic code of an organism (the "genome") as the source of these unique signatures. Other sensors, including many that are now fielded, use a kind of recognition between two molecules that is like a lock and key. The most commonly used of these interactions is the attachment of an antibody to its target molecule.

Given the diversity of living things on Earth and the enormous variety of unique molecules that comprise them, scientists have looked to nature for examples of effective biological recognition molecules (BRMs) derived from natural processes. The immune system of mammals is one such example; they can produce between 100 million and 1 billion different antibodies. Antibodies are BRMs, each of which binds to a small molecular shape different from any other. Animals exposed to a foreign microbe (immunized) produce an abundance of antibodies specific for the invading organism.

Antibodies from immunized animals have been isolated and bound to fiber optics, silicon chips, and other nonbiological surfaces to create "biosensors," hybrid devices that detect target molecules by com-

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bining biological molecules such as BRMs with electronic or optical microsensors. The binding of the target molecule to the sensor-bound BRM creates a measurable physical change that registers as a signal in the sensor device, but only if the presence of the target molecule is recognized by its specific binding to the immobilized BRM. Our work focuses on next-generation BRMs—molecules beyond conventional antibodies—and includes both molecules derived from biotic (natural biological) and biomimetic (synthetics that mimic biological molecules) approaches.

Current Solutions

It is important to stress that BRMs (in this case, antibodies) are at the heart of all currently fielded BW agent detection systems; without the antibodies, there is no BW agent detection. Two technologies are used to produce antibodies for biological defense. The "polyclonal" method produces a mixture of antibodies that is harvested from the blood serum of animals repeatedly injected (immunized) with a "disarmed" BW agent. The "monoclonal" method produces individual antibodies, each produced by cells in culture flasks.

The Joint Program Office for Biologial Defense (JPO-BD), through its Critical Reagent Program, is the agency responsible for acquiring antibodies that recognize BW agents, ensuring their quality, and providing them to the vendors that produce biosensors. In this way, the JPO-BD supports several sensor platforms that incorporate antibody-based biosensors, including the Joint Biological Integrated Detection System, the Joint Biological Point Detection System, and Portal Shield. This work also ensures that the required quantity and quality of antibodies are available to meet fielding requirements and support continued research, development, test, and evaluation efforts.

The U.S. Army Soldier and Biological Chemical Command's Edgewood Chemical Biological Center (ECBC), Aberdeen Proving Ground, MD, is the home of JPO-BD's Critical Reagent Repository (CRR) for antibodies. The CRR includes sophisticated storage facilities and validation labs and maintains a highly detailed tracking database of all antibodies being used in DOD biological defense efforts. ECBC staff scientists, in addition to maintaining the repository, are developing new BRMs and new applications to supplement and possibly replace currently fielded reagents.

Recombinant Antibodies

There is considerable lot-to-lot variability in the current production of antibodies. This is especially true for polyclonal antibodies because the individual response of each animal to an agent can vary dramatically. The process of producing antibodies in animals or in a mammalian cell culture is also time-consuming, which limits the capacity for "just-in-time" or surge production in time of conflict.

A recent advance in antibody production technology is the cloning of antibody genes from mammalian cells in large quantities, or "libraries" of genes. These genes have been introduced into bacteria, and the resulting population of bacteria can be rapidly sorted to obtain those making antibodies that have the desired qualities of specificity for a BW target and sensitivity for its detection. This technology has been proven capable of producing antibodies for BW agent detection that are of excellent quality and greater uniformity from lot to lot; therefore, their inclusion in fielded bioassays will result in greater reliability. Recombinant antibodies are also faster and less expensive to produce and acquire in quantity; therefore, establishing a process for their production can improve the maintainability and supportability of fielded biodetection systems. A number of these valuable reagents are currently under development at ECBC, and we have begun to explore this technology for eventual sourcing of several defense-critical antibodies for biodetection.

Recombinant Peptides

Whole antibodies are relatively large, as molecules go. However, that portion of the antibody that actually interacts with the target is relatively small—perhaps 1 percent of the antibody actually contacts the target molecule. Most of the rest interacts with the animal's immune system, a function that is not required for use in detecting a BW agent. Portions of the antibody that specifically bind the target are at the ends of the two "arms" of the molecule, and their smallness has prompted scientists to ask, "Can only those short pieces of antibody protein, or *peptides*, substitute as BRMs for the whole antibody?"

Just as libraries of antibody genes have been cloned and sorted, recombinant genetic technologies have enabled scientists to create and sort collections of genes that encode short proteins, or peptides, finding those that bind to a molecule of interest. A library composed of tens of millions of random peptide sequences can be rapidly screened against a specific threat agent to identify a peptide that mimics the activity of a whole antibody. Also, like the recombinant antibodies, peptides can be made in bacterial cells and be harvested from fermentations, or they can be produced in large quantities entirely by chemical synthesis. These peptide libraries are called "combinatorial" because of the randomness of the peptide-encoding genes.

An additional advantage of screening for peptide mimics is that the search is not limited to immunogenic binding sites. Recombinant antibodies are often made using genes cloned from animals. Animals will not make antibodies effectively against some molecules, partly as a protection against attacking their own bodies. Peptides, however, can be selected for binding to other molecular features of the threat agent that do not elicit an immunogenic response, thus allowing for a potentially greater diversity of BRMs to be discovered.

Improved Logistics

The search for recombinant antibodies and peptides that mimic whole antibodies offers several technical and logistic advantages in production and QA/QC issues compared to conventional methods of production in animals and mammalian cell culture. First, the combinatorial libraries, once formed, can be rapidly screened and candidate molecules selected for further test and development within 1 to 2 weeks of the start. Conventional antibody development, as noted previously, is much more time-consuming. Second, the libraries present an efficient means for rapidly identifying reagents that recognize emerging threat agents for which no current antibody exists, or for live pathogens that are too lethal to effectively use animal inoculations. Third, recombinant antibodies and peptides are smaller than whole antibodies and therefore more stable outside controlled environments. Smaller molecules afford greater control in orienting and immobilizing the molecules on a sensor platform (e.g., microchips) with greater assurance that the active binding site is fully exposed to sample media. Fourth, once an antibody fragment or peptide has been identified with strong binding properties, the molecule can be further engineered by directed mutation or specific amino acid substitution to "fine tune" its specificity or affinity for the target agents. Finally, recombinant antibodies and peptides can be produced in large quantities within rigidly controlled manufacturing processes that yield cost savings from economies of scale while achieving higher levels of QA/QC between different production lots.

The Army's Role

ECBC's Process Engineering Facility (PEF) is DOD's sole scale-up PEF dedicated to the research, development, and validation of manufacturing processes for producing biological materials. The PEF staff emphasizes cooperation between basic molecular biology research scientists and the bioprocess engineers responsible for production and purification of antibodies and other products. The molecular biology laboratory in which recombinant antibodies and peptides are cloned is the same building in which they are produced, tested to meet QA/QC requirements, and stored in the CRR, thus ensuring a continuous transition of basic research and development (R&D) into advanced development and final production. Thus, DOD benefits by having the logistic advantage of housing a repository of BW detection materials within a facility with strong R&D and end-stage production capabilities.

Conclusion

Molecular engineering of recombinant antibodies and peptides provides unique opportunities for improving the production of current reagents, developing new reagents, and reducing the life-cycle costs associated with reagent production and continuous process improvements. Life-cycle management of critical reagents used to detect and identify biological agents extends from the support of basic R&D of new or better reagents; to the scale-up production, testing, and validation of the reagents in field trials; to sustained production of quality reagents to the tri-Service user community.

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